ENDAMŒBA CITELLI SP. NOV. FROM THE STRIPED GROUND SQUIRREL *CITELLUS TRIDECEMLINEATUS*, AND THE LIFE-HISTORY OF ITS PARASITE, *SPHÆRITA ENDAMŒBÆ* SP. NOV.

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ENDAMŒBA CITELLI sp. nov.

In the autumn of 1925 the writer made microscopic examinations of the fæcal mass from the intestines of eight ground squirrels belonging to the species *Citellus tridecemlineatus*. These animals were captured alive at various times in the vicinity of Ames, Iowa, brought to the laboratory, and examined at once for parasitic protozoa. In addition to a number of other interesting protozoa, six of the ground squirrels were found to harbor amœbæ in their cœca. In four of the animals the amœbæ were extremely rare, but two showed extremely heavy infections. The movements of the live amœbæ were studied in normal saline solution, and permanent mounts were made by the well-known Schaudinn-iron-hæmatoxylin method.

The amœbæ live within their host in association with myriads of other protozoa. The habitat is limited to the cœcum and the part of the colon immediately adjoining. Localization in the cœcum has been noted by Kessel (1924) in the case of several species of rat and mouse amœbæ. Kessel thinks the PH relationship has something to do with this localization of habitat. Perhaps another factor is the more fluid content of the cœcum which makes a more favorable medium for amœbæ than the colon, where the water content is very much reduced by absorption.

Examination of the substances in the food vacuoles of the amœbæ show that they feed largely upon bacteria and nondescript particles of undigested vegetable matter. The food vacuoles and cytoplasm often contain a curious parasite of the amœba which resists digestion. This will be discussed below. No red blood corpuscles or tissue cells were ever observed within the cytoplasm. These facts make it fairly safe to conclude that the amœba is a commensal and not a true parasite.

The locomotion of the free forms was studied, because the character of pseudopod formation has come to be of so much importance in correct classification (Dobell, 1921, Kofoid, Swezy, and Kessel, 1923). When kept slightly warmed they exhibited great activity with frequent pseudopod formation. The pseudopods were clear and broadly rounded. The endoplasm did not invade them after they were formed, but they were often withdrawn before the endoplasm had completely filled them. They were not, however, thrust out with the explosive suddenness characteristic of *Endamœba histolytica* which the writer has been fortunate enough to observe on several occasions. Typical "fountain streaming" or limax movement was observed in a number of individuals. The nucleus is not prominently visible in the living specimens, but it can be made out after observing the amœba carefully for a while.

Measurements of stained specimens of the free amœbæ show considerable variation. The smallest one measured eleven by ten micra, and the largest twenty-three by twenty-five micra. An average of ten amœbæ measured was fifteen by sixteen micra, which is somewhat smaller than either *Endamœba coli* or *Endamœba histolytica*, but compares favorably with Kessel's (1924) measurements of *Councilmania decumani* from mice and rats. The nuclei of the free forms measure from four by four micra to six and one tenth by five and two tenths micra in size. Ten measurements averaged four and nine tenths by four and eight tenths micra, which is slightly smaller than the figures given for *E. histolytica* and *E. coli*. (Hegner and Taliaferro, 1924.)

The nucleus is definitely of the vesicular type, with a deeply staining karyosome which varies in position from central (Fig. 6) to extremely excentric (Fig. 1). Usually it is less pronouncedly excentric, as in Fig. 2. This karyosome is surrounded by a clear achromatic zone, which in turn is surrounded by a layer of more or less concentrated slightly basophilic substance (Figs. 1–6; 8). Between this layer and the nuclear membrane is an achromatic reticulum on which are suspended fine basophilic granules. The achromatic nuclear membrane is encrusted on the inner surface by a fine beading of chromatin granules, which is intermediate in coarseness between *E. histolytica* and *E. coli.* A few binucleate individuals were found, but no division figures.

Cysts were exceedingly rare, and only four eight-nucleate cysts could be found; but these were well stained and suitable for study. The shape is nearly spherical (Fig. 9); size, fifteen and one half micra in diameter. Their especially characteristic feature was the thickness of the cyst wall, which was in all cases about one micron. This compares with a thickness of less than 0.5 μ in *E. histolytica* and *E. coli.* The nuclei likewise were fundamentally different from those of the other *Endamœbæ.* All the chromatin from the karyosome and periphery of the nucleus appeared to have collected into a number of irregular, deep-staining blobs. Some of these lay upon the nuclear membrane, while others were farther in the interior of the nucleus. The nuclei of the cyst measured about 2.8 micra in diameter. In contrast to the free forms, where it is coarsely alveolar, the cytoplasm of the cyst appeared granular. Chromatoid bodies were absent, except for a few small dark staining splinter-like bodies in the center of the cyst. The developmental stages of the cyst were not found.

It was upon the basis of thickness of the wall of the cysts, its yellowish tinge, and the character of the cyst nuclei that a new species was created for this amœba. Were it not for this characteristic cyst, it would be difficult to distinguish this from many other *Endamœbæ; e.g., E. muris* (Grassi, 1882) of the mouse and rat.

The writer in a previous paper (1922) pointed out that simply finding a parasite in a host where it had not previously been found was no valid reason for considering it a new species. Especially is this true in the case of amœbæ; for if, as Kessel (1923) determined in his experiments, rodents can be infected with the human amœbæ, it is probable that man can be infected with the amœbæ of rodents. Two points are so sufficiently clear that they should not be ignored in future work in amœbæ. First, is the species being considered sufficiently different morphologically from closely related species so that it should be considered a new one? Second, there is the possibility of man, or domestic animals, becoming the host of an endamœbæ normally found in lower animals. Kessel's work on specificity has encountered severe censure, especially from European workers (*e.g.*, Wenyon). It seems to the writer that the more logical method to criticize the work would be to repeat it, and thus determine if it is really open to such serious defects as has been charged.

Sphærita endamœbæ sp. nov.

The name *Sphærita* was given by Dangeard (1886) to a genus of the family Chytridiaceæ, which he considers to represent a transition from animal toward plant forms. Likewise, Doflein (1916) assigns these forms to the borderline between the plant and animal kingdoms, with the additional comment that they must be reinvestigated by one who would study the relationships of the sporozoa, flagellates, and rhizopods. They are of interest to protozoölogists not only because of their phylogenetic relationships to the protozoa, but also because *Sphærita*, and other cythridines, such as *Nucleophaga* (Dangeard, 1895), are parasites upon protozoa, *Sphærita* in the cytoplasm, and *Nucleophaga* in the nucleus. A number of the earlier observers, particularly Stein, Carter, Kent, and de Lanessan misinterpreted the developmental phases of a *Sphærita* within *Euglena* as the production of embryos from the nucleus of the *Euglena*, which grew flagella and later developed into the adult flagellate (see Dangeard, 1886). Dangeard (1886, 1894, 1895) clearly showed that what these authors considered to be the growing and multiplying nuclei of *Euglena* were in reality cythridine parasites of the flagellate for which he established the genus *Sphærita*.

There are not many references to *Sphærita* in the literature. Dangeard proposed the name *Sphærita endogena* for the form found in flagellates (*Euglena*, etc.) and rhizopods (*Nuclearia* and *Heterophrys*). Later Chatton and Brodsky (1909) proposed a separation of the species found in these two groups of protozoa, suggesting that *Sphærita endogena* be retained for the form found in rhizopods, and that the *Euglena* parasite be called *Sphærita dangeardi*. Chatton and Brodsky (1909) described a

Sphærita from *Amœba limax* Dug., which they found to be different morphologically in the younger developmental stages from *S. dangeardi*. No comparison was made with *S. endogena*. Dobell (1919) mentions a *Sphærita* in the free forms of the parasitic amœba, *Endolimax nana*. Kessel (1924) found a *Sphærita* in *Councilmania muris*, entozoic in mice and rats. There are a number of other papers on *Nucleophaga*, closely related to *Sphærita*, except that it is found in the nucleus. It was originally described from *Amœba verrucosa* by Dangeard (1895), but we will not discuss this genus any further here.

The greater number of *Endamœba citelli* from one ground squirrel were parasitized by a species of *Sphærita*. This material showed so great an abundance of individuals in different stages of development that it has been possible to follow almost the complete life-cycle of this interesting cytozoic organism. The earlier stages of development of the parasite were the first to be seen within the cytoplasm of the amœba (Figs. 2, 3, 4). The first impression was that they represented nuclei in the process of construction from chromidia. Further search revealed the large plasmodia with maturing spores (Fig. 7), which led to the correct identification of the bodies as stages of the life-cycle of a *Sphærita*. It would not be surprising if intracellular parasites of protozoa have led observers astray more often than is generally known; *e.g.*, Leidy in Plate VII. of his "Fresh-water Rhizopods of North America" figures a number of specimens of *Amœba villosa* with "large and coarsely granular nuclei," which "nuclei" were probably typical sporangia of a *Sphærita*, the "coarse uniform granules" being the spores. This interpretation is strengthened by Fig. 15 of the same plate, which Leidy describes as representing "collapse of the contractile vacuole and the bursting of one of the nuclei with the simultaneous escape of the granules or spores of the nucleus and the contents of the contractile vacuole." What he probably observed was the liberation of the spores from a sporangium of *Sphærita*.

The life-cycle of *Sphærita citelli* can perhaps best be described by referring frequently to the figures of the plate. Fig. 2 represents an amœba with two parasites in the earlier stages of development. The lower one has a fine cell membrane, which encloses a centrally located, deeply-staining nucleus. The nucleus shows no nuclear membrane or other differentiation, and its diameter is about two thirds that of the cell. The nucleus of the upper specimen has just divided with no apparent spindle or attraction spheres. At this stage the opposing surfaces of the two nuclei are flattened, with the remaining surface of each nucleus convex. The uninucleate and binucleate stages of the cell are about the same size, measuring from 1.9 to 2.5 micra.

A second bipartate division provides the plasmodium with four nuclei with the planes of both divisions still plainly marked (Figs. 3, 4, 18). The organism has become more oval in shape and has increased in size to about 2.5 micra in width and from 2.8 to 3.3 micra in width. From this stage the divisions of the nuclei are not necessarily simultaneous. Specimens were found with eight nuclei (Fig. 5), or with six nuclei, four of them smaller and resulting from the division of two of the nuclei of the four-cell stage, with the other two larger and still undivided (Fig. 19). The plasmodium at this stage measures from 3.7 to 4.0 micra in width to from 4.2 to 5.3 micra in width. Divisions are multiplied until the multi-nucleate spherical stage is attained (Figs. 6, 20, 21). These spheres measure from 5.3 to 8.8 micra in diameter.

The nuclei of the spheres just described stain uniformly black. The next stage in the cycle is the transformation of these nuclei into spores. In this process they enlarge somewhat, stain less intensely, and form a definite spore wall (Figs. 7, 22). Some of them show a thickening of the wall on one side (Fig. 22). These spores usually vary in size from 1.0 to 1.6 micra in diameter. The larger spore in Fig. 22 is exceptionally large, measuring about 1.8 micra. Occasionally spores no larger than 0.5 micron in diameter are found.

A comparison of *Sphærita endamœbæ* with the *Sphærita* from *Amœba limax* so carefully described by Chatton and Brodsky (1909) shows certain fundamental differences. First, the nuclei of the young uninucleate forms are comparatively large and central in *S. endamœbæ*. Those from *Amœba limax* were punctiform and excentric. These distinctions alone are sufficient to justify a distinction between the two species. Second, not all the nuclei of *S. endamœbæ* develop simultaneously into spores, as they apparently do in the form from *A. limax*. Third, there is no nucleus present within the spores of *S. endamœbæ*, although the contents of the spore are clearly visible. Chatton and Brodsky state that it was difficult to see the interior of the spores of their *Sphærita* even in stained specimens. In a few cases, however, they observed an excentric nucleus within the spore. Fourth, the appearances of dividing nuclei of the multinucleate plasmodium dffer in the two species. Those of *S. endamœbæ* are bilobed, or dumb-bell shaped. Those described by Chatton and Brodsky presented the appearance of two cuneiform polar caps. Measurements of the two species in various stages lie within approximately the same limits. The above stated facts make it evident that the *Sphærita* of the *Endamœbæ* is altogether different from that of the free-living amœba, *A. limax*.

Likewise I believe it is different from the one figured by Dobell (1919) in *Endolimax nana*, an amœba entozoic in man. If his figures be correct, the nucleus of the uninucleate stage is punctiform and excentric, as in the parasite of *A. limax*. The morula-shaped mass of spores is likewise not characteristic of *S. endamœbæ*. Kessel's (1924) account is too meagre to afford a comparison.

Chatton and Jansky were not able to determine whether the spores of their *Sphærita* became flagellated, after the manner of the zoöspores of *Sphærita endogena* and *S. dangeardi* as described in the accounts of Dangeard, or remained immobile and were passively ingested by the amœba. Although actual reinfection by the spores was not observed, the writer has been able to follow out the process in his prepared slides.

Among the bacteria present on the slide one occasionally finds dumb-bell shaped organisms, resembling *Azotobacter*. There seem to be two general sizes, one considerably larger than the other (Fig. 10). These multiply by binary fission (Fig. 11). It is not unusual to find these dumb-bell-shaped bacteria-like bodies in the food vacuoles of the amœbæ (Figs. 8, 12, 13). In the food vacuoles the organism undergoes considerable change. Dense granulation appears in the more or less homogeneous cytoplasm. The dark granules collect in a deeply-staining clump in the center of the cell (Figs. 3, 8, 14). The two members of the dumb-bell shaped pair usually separate, and are carried some distance from each other. Finally, the deeply-staining mass of granules becomes compact and uniformly solid (Figs. 2, 3, 8, 15, 16, etc.). By this time the fluid content of the vacuole has disappeared and the wall of the *Sphærita* is contiguous with the cytoplasm of the amœba. The development then proceeds as described above.

The writer realizes that it may be objected that two organisms have been confused in this cycle, and that what has been interpreted as the infective form of the *Sphærita* is in reality a bacterium ingested as food. This danger was a cause of considerable anxiety, and it was not until a large amount of material was studied that the writer was convinced of the specific identity of the two forms. A careful study of such appearances as in Fig. 8 (where one finds a perfect series from the bacterium-like form to the early uninucleate form, unmistakably that of *Sphærita*) brings conviction of the transition from one form to the other. The only gap in the life-history is the failure to observe convincing stages of the growth of the spores into these larger bacterioid forms. It is to be expected that such stages would be exceedingly difficult to find, considering the amount of the fæcal mass in proportion to the number of spores. The writer has observed, however, the smaller dumb-bell-shaped dividing spores resembling the smaller individual in Fig. 10 within an old sporangium from which all but a few of the spores had been expelled.

The extracellular development of *S. endamœbæ* differs in several important respects from that described by Dangeard for the forms which he found in free-living flagellates and rhizopods. Here the zoöspores became elongated and flagellated as they left the sporangium. Then they united in pairs, as in conjugation. The spores studied by the writer were never flagellated when found outside the amœba, and no conjugation of spores was observed, although what appeared to be dividing spores indicating a free multiplication cycle were often found.

As in the case of *Sphærita* living in other protozoa, this *Sphærita* is mildly pathogenic to its host. Most parasitized amœbæ exhibit no degenerative changes of any kind (Figs. 2–6, 8). Some of the more heavily infected ones, however, manifest the ill-effects of parasitism by abnormal nuclear appearances (Fig. 7). The karyosome becomes swollen and irregular in shape.

The chromatin beading on the nuclear membrane collects into thick, elongated, deeply-staining blobs. Dobell (1919) also figures nuclear degeneration in parasitized *Endolimax nana*.

Summary.

1. *Endamœba citelli* sp. nov. is a commensal in the cœcum of the striped ground squirrel, *Citellus tridecemlineatus*.

2. The nucleus of the free form is typical of the genus *Endamœba*.

3. The cyst is characterized by a nuclear structure somewhat different from that known for other amœbæ, and an unusually thick cyst wall with a yellowish refraction.

4. The cytoplasm of this amœba may contain a cytridine parasite, *Sphærita endamœbæ* sp. nov.

5. The developmental cycle of this cytozoic parasite was followed from the free bacterium-like infective stage to the spore liberated from the sporangium inside the cytoplasm of the amœba.

6. *Sphærita endamœbæ*, like other members of the genus, produces degenerative changes in the protoplasm of its host, particularly in the nucleus.

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EXPLANATION OF PLATE.

× 1420.

FIG. 1. Free form of *Endamœba citelli* with typical nucleus fixed with two pseudopods extended. Two food vacuoles in cytoplasm contain respectively a bacterium and a partially digested yeast.

FIG. 2. Amœba infected with young uninucleate and binucleate *Sphærita*.

FIG. 3. Amœba with same stages plus one younger individual with nucleus in process of formation and an individual with four nuclei.

FIG. 4. Large amœba with one parasite having four nuclei.

FIG. 5. *Sphærita* has eight nuclei, each dumb-bell-shaped preparatory to division.

FIG. 6. Multinucleate *Sphærita* in cytoplasm of amœba.

FIG. 7. Large sporangium with nuclei developing into spores.

FIG. 8. Amœba with large food vacuole containing infective bacterium-like stages developing into typical uninucleate cytozoic forms.

FIG. 9. The eight-nucleate cyst of *E. citelli*.

FIGS. 10–11. Free bacterium-like stages of *Sphærita endamœbæ*.

FIGS. 12–14. Bacterium-like stages in food vacuoles of the amœba.

FIGS. 15–22. Developmental cycle of *Sphærita* in cytoplasm of amœba. Fig. 16 does not represent a division of the organism in Fig. 15, but rather the same stage of development, the next stage after Fig. 14. The two members of the pair later separate.